Author's response to reviews

Title: DPOFA, a Cl-/HCO3- Exchanger Antagonist, Stimulates Fluid Absorption Across Basolateral Surface of the Retinal Pigment Epithelium.

Authors:

Pavel Iserovich (pi3@columbia.edu)
Qiong Qin (qq2101@columbia.edu)
Konstantin Petrukhin (kep4@columbia.edu)

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Author's response to reviews: see over
Dear Ms. Aimé,

We are submitting the second revision of the manuscript entitled “DPOFA, a Cl-/HCO₃⁻ Exchanger Antagonist, Stimulates Fluid Absorption Across Basolateral Surface of the Retinal Pigment Epithelium”.

In response to the reviewers’ criticism we introduced minor edits to the manuscript while providing a point-by-point response to the reviewers’ criticism the section that follows this page.

In our point-by-point response to the reviewers’ criticism reviewers’ critiques are shown as underlined blue text. When the new text added to the revised version is cited in the response, it is highlightened in yellow.

New sections of the revised manuscript are highlightened in yellow. Deleted portions of the manuscript are shown as strikethrough text.

Thank you very much for your consideration, and I appreciate the opportunity to submit our paper to your journal.

Sincerely,

Konstantin Petrukhin, Ph.D.
Associate Professor
Department of Ophthalmology
Columbia University Medical Center
Response to Reviewers

Reviewer #1/ Antje Wurm.

1. Unfortunately, I do not have any access to the first version, thus, I am not sure about the old data.

The resubmitted version of the manuscript contains all the portions of the original draft. The deleted paragraphs that were present in the original submission but absent from the revised version are shown as a crossed out text. The added portions that were not present in the original submission are highlighted in yellow. We provided the explanation on text marking in the cover letter that was accompanying the revised manuscript.

2. …the authors presented data using concentrations of 6, 20, and 60 µM and time points after 5, 40, and 65 minutes. Now, they used concentrations between 1 and 20 µM and time points of 10, 15, 20, and 30 minutes. Thus, there is, at least partly, no overlap of both data sets. It seems as if the authors always exclude a part of their data from presentation.

As the difference between the 6, 20 and 60 µM DPOFA concentrations did not reach statistical significance, we decided to exclude the DPOFA titration data in the fluid absorption model from the revised manuscript; Figure 3 from the original submission that compares the 6, 20 and 60 µM DPOFA concentrations is removed. Our response to the reviewer’s comment (It seems as if the authors … exclude a part of their data from presentation) is YES, we did remove from the publication the data that was not supported by the statistical significance analysis. In the revised manuscript we report the only conclusion that can be supported by the statistical significance analysis. This conclusion is as follows: when added in 1-20 µM concentrations, DPOFA significantly (p=0.013) increased water absorption within first 20 minutes by $3.22 \pm 1.4 \, \mu l/cm^2h$ while vehicle control decreased absorption by $2.1 \pm 1.39 \, \mu l/cm^2h$ (Revised Table 1 and Revised Figure 3). This is the only result concerning the DPOFA effect on fluid absorption that we are presenting in the revised manuscript. No experimental datapoints that fit the description of the selected subgroup (DPOFA concentrations within the 1-20 µM; time after drug addition within the first 20 min) were excluded from the analysis. The subset of datapoints from the DPOFA titrations comparing 6, 20 and 60 µM DPOFA concentrations (originally presented in Fig 3 of the first draft) that fit the description of the selected subgroup (namely, the datapoints related to DPOFA concentrations within the 1-20 µM range and timepoints after drug addition within the first 20 min) are also included in the pool of data contributing to the analysis describe in the second revision of the manuscript.

3. The other possibility is that new experiments were done, however, the authors claim that this was not possible because the substance was lacking.

No additional experiments have been performed

4. In any case and independent on the old version, the new idea in the revised version was to summon up the data obtained from experiments with the different concentrations (1-20 µM).
This is the correct statement. We eliminated from the revised version all the data that could not be supported by the statistical significance analysis. We focused on the analysis of the subgroup of concentrations and timepoints for which the difference with the compound vehicle could be established with the statistical significance.

5. Next step is that they authors pooled data from the first 20 minutes (but excluded the values from 30 minutes) to get a significant effect versus control.

Table 1 contains the 30 min data. However, the statistical significance analysis supports only one conclusion: when added in 1-20 μM concentrations, DPOFA significantly (p=0.013) increased water absorption within first 20 minutes by 3.22 ± 1.4 μl/cm²·h while vehicle control decreased absorption by 2.1 ± 1.39 μl/cm²·h (Revised Table 1 and Revised Figure 3). We limit the scope of the publication to reporting the data that can be supported by the statistical significance analysis.

6. In contrast, the authors stated in the old version that the drug was most effective at 40 min post dosing.

This statement is incorrect. We never claimed in the first version of the manuscript that the drug is most active at 40 min post-dosing.

7. In the new version, no data after 40 min are presented at all. What is the rationale to restrict the data to values obtained till 30 minutes after application in the revised version of the paper?

We limit the scope of the publication to reporting the data that can be supported by the statistical significance analysis. This is the rationale for not presenting the data that is not supported by the statistical significance analysis in the revised draft of the manuscript. We don’t want to speculate on biological reasons for no difference between the drug and the vehicle at the 40 min timepoint after the drug addition.

8. Additionally, the way of statistical evaluation of the data appears questionable or at least is not delineated sufficiently to understand correctly. Considering the numbers given in table 1 one can just speculate that experiments with the different concentration were not repeated to a sufficient amount also in respect of statistical analysis. However, it does not seem reasonable to overcome this drawback by simply pooling these data.

In this manuscript we present the evidence in support that at lower concentrations DPOFA affects water absorption through the bovine choroid-RPE explant. The subset of drug concentrations is different in respect of the stimulation of water absorption from the vehicle at a subset of timepoints following the drug addition. This data is supported by the statistical significance analysis.

9. In the letter to the editors, the authors claim to have reduced the scope of the manuscript to reporting two findings supported by statistical analysis which includes the finding that there is no significant difference in the effect among the tested concentrations. In fact, although mentioned in the results section there exist no conclusive data in the present form of the paper that supports this
In the revised manuscript we report the only conclusion that can be supported by the statistical significance analysis. This conclusion is as follows: when added in 1-20 µM concentrations, DPOFA significantly (p=0.013) increased water absorption within first 20 minutes by 3.22 ± 1.4 µl/cm²h while vehicle control decreased absorption by 2.1 ± 1.39 µl/cm²h (Revised Table 1 and Revised Figure 3). This is the only result concerning the DPOFA effect on fluid absorption that we are presenting in the revised manuscript.

10. Finally, this kind of approach seems like an alchemistic experiment: Take any concentration between 1 and 20 µM, wait for any time between 5 and 20 minutes and you will have an effect. This brings up several questions for the unbiased reader, such as why are there no data about effects of exact concentrations – was it impossible to apply distinct concentrations for whatever reason? To my mind, such kind of data presentation is against the rule of good scientific practice and should not be published.

Post hoc subgroup analysis following experimentation in the complex biological systems is a common practice. The outcomes of most clinical trials (e.g., AREDS) are analyzed in this way. While we tested DPOFA at multiple concentrations and timepoints, we found a statistically significant difference between DPOFA and placebo only when we analyzed the drug effect in a subset of drug concentrations (1-20 µM) at a subset of timepoints. This is the finding that we would like to report.

10. Minor Essential Revisions
Results section
35th line ...with the affect ... correct to ...with the effect...
Figure legends
Figure 2.
4th line ... and bovine (C) isoform... should be corrected to ...and bovine (D) isoform...

Requested corrections made.

11. Figure 3
If there exists any significant difference between treated RPE and control tissue, please mark with the respective symbols directly in the graph for easier understanding of the reader. The way it is described in the actual paper apparently is delusive.

While we disagree with the statement that the figure legend is “delusive”, the following changes are made to clarify the point and avoid misinterpretation.

Figure 3. Comparison of changes in water pumping rates in bovine RPE-choroid explants induced by DPOFA and vehicle treatment. DPOFA at 1-20 µM concentrations was added to apical and basal baths of the chamber. The number of experiments and change in pumping rate values are shown in Table 1. Mean values are plotted with error bars depicting Standard Error of Means (SEM). The change in
pumping rate from apical to basal side of the RPE-choroid explant was calculated at 10, 15, 20, and 30 minutes after drug and vehicle addition. While no statistically significant difference between compound doses could be discerned, we were able to detect the statistically significant (p=0.013) increase in fluid absorption after drug treatment versus vehicle control during the first 20 minutes post drug/vehicle addition when the data for all DPOFA concentrations within the 1-20 µM range were pooled for the analysis. When compared with the vehicle, DPOFA increased water absorption within first 20 minutes after drug treatment in a statistically significant way (p=0.013).
Reviewer #3/ Dongli Yang.

As minor essential revisions, I would suggest that the authors combine Figure 3 and Table 1 as the data in Figure 3 and Table 1 are the same. For example, add n (number of experiments) or means ± SEM (n) above or below each bar in Figure 3, and modify Figure 3 legend accordingly.

Our response to the critical comment from another reviewer caused a substantial lengthening of the Figure 3 legend. While combining Figure 3 with Table 1 is a reasonable suggestion, it may require additional lengthening of the legend thus further overloading it at the cost of the lucidity.

Other minor essential revisions:
1. Abstract: I would suggest using RPE instead of retinal pigment epithelium the last sentence in the Background section as RPE is spelled out in the first sentence of this section.

The requested change made.

2. Introduction: “Pharmacological up-regulation of fluid reabsorption from subretinal space to choroid across the retinal pigment epithelium has been suggested as potential treatment strategy for retinal detachment [5].” I would suggest changing “…retinal pigment epithelium …” to “… retinal pigment epithelium (RPE)…”.

The requested change made


The requested changes made.

4. Figure legends: Figure 2, “Panels B and D shows the results of control PCR amplification with human (B) and bovine (C) isoform-specific primers …” Need to change “…bovine (C)…” to “…bovine (D)…”

The requested change made